International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 4, Issue 1, Feb 2013, 89-100

A COMPARISON BETWEEN CARBENDAZIM FUNGICIDE AND *TALAROMYCES FLAVUS* IN CONTROLING VERTICILLIUM WILT OF POTATO UNDER FIELD CONDITIONS

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ABSTRACT

Potato Verticillium wilt, caused by *Verticillium dahliae* and *V. albo-atrum*, possess specific importance and one of its control methods is biological control using antagonistic fungi. In this study, three isolates of *Talaromyces flavus* (TF-Po-V-49, TF-Po-V-50 and TF-Po-V-52) with the most inhibitory effect on Verticillium wilt disease in the greenhouse were selected for evaluation in the field. Rice bran was used for the preparation of different inoculums affected by the above-mentioned isolates. For application in the field, potato tubers were coated with tree different inoculums. During 2011 and 2012, field experiments were carried out in randomized complete block design with five treatments and four replications in the agricultural research station of Hamedan province of Iran. Treatments included: 1-3: each inocolum affected by one of three isolates of *T. flavus*; 4: Carbendazim fungicide and 5: control without inoculum and fungicide. According to the results of this study, TF-Po-V-52 was the most effective fungal antagonist in decreasing Verticillium wilt disease severity and incidence (18.04 and 40.31% respectively) and increasing yield of potato (17.48%) and could be a potential biofungicide and biofertilizer in the fields. Statistical groups of the treatments in terms of average yield showed that the yield was increased from 3.69 to 26.27% in all treatments compared to the control. Among all treatments, those affected by carbendazim and TF-Po-V-52 showed the most efficacy in increasing yield (28.74 and 26.74 ton/ha respectively) in comparison with the control (22.76 ton/ha).

KEYWORDS: Biological Control, Potato, *Talaromyces flavus*, *Verticillium albo-atrum*, *Verticillium dahliae*

INTRODUCTION

According to the latest available information, the area of fields under potato cultivation in Iran is about 189 thousand hectares with the average annual production of 4.8 million tons (Felenji and Ahhmadizadeh, 2011). Verticillium wilt is one of the most important yield reducing factors in potato fields of Iran. The host range of this pathogenic fungus is very wide and covers about 160 species from 40 families of different plants. The losses caused by Verticillium wilt disease are very high since it may lead the tubers never forms in the plant (Bhat and Subbaro, 1999). Verticillium wilt disease has inflicted noticeable damage to potato crop where 30 percents of the agents for this disease belonged to Verticillium isolates including *V. dahliae* and *V. albo-atrum* (Saremi and Amiri, 2010).

Previous studies have shown that in the area with the partial variations in environmental factors like reduced temperature and increase in the rate of soil nitrogen compounds through increase in chitinase enzyme, *V. albo-atrum* may cause severe disease in the crop plants (Roberts, 1943; Thanassoulopouos and Hooker, 1968; Sahai and Manocha, 1993). The infection percent of this disease for potato crop has been reported as high as 70 percent in sensitive cultivars, 45-50%

for cultivation prevalent variety and 5-15% for resistant cultivars, indicating the importance of its control that has been discussed within many studies (Busch, 1996; Krikun and Orion, 1979; Sampson, 1980; Powelson and Rowe, 1993; Nobel and Roberts, 2004).

T. flavus as an antagonistic fungus in Iran was isolated and identified for the first time from the soil and rhizosphere of cotton fields in Iran (Naraghi *et al*., 2003). The results of experimental investigations regarding review on the impacts of inhibitory mechanisms of *T. flavus* on growth of soil-borne pathogenic factors among several crop plants have shown that several mechanisms including mycoparasitism, production of volatile and non- volatile compounds is employed by this fungus, from which production of non- volatile metabolites seems to be the most important in inhibiting and reducing the growth of cotton Verticillium wilt disease and its causal agent (Naraghi *et al*., 2003).

In a similar study, it was also indicated that there were some common antagonistic mechanisms among different isolates of *T. flavus* regarding three crop plants including potato, tomato, and greenhouse cucumber, which were mycoparasitism, and production of volatile metabolites against *V. albo-atrum*, mycoparasitism against *F. oxysporum*, and producing non- volatile compounds against *R. solani* (Naraghi *et al*., 2012b).

Pursuant to the above studies, the greenhouse and field- related investigations regarding the possible biologic control of Verticillium wilt disease on cotton and sugar beet root rot by means of biological fungicide affected by *T. flavus* while the results indicated that in addition to significant reduction in disease index, this fungus has caused significant premature increase and rising yield (Naraghi *et al*., 2004, 2006, 2008, 2012a, 2012b). Likewise, the results from greenhouse experiments on biologic control of Verticillium wilt disease in potato, tomato, and greenhouse cucumber caused by *V. albo- atrum* by several isolates of *T. flavus* have indicated that these isolates have significantly reduced disease index and increased growth factors including root length, length of ring, height, and fresh and dry weight in above plants (Naraghi, 2010; Naraghi *et al*., 2010a, 2010b; Naraghi *et al*., 2012b, 2012c, 2012d).

Therefore, with respect to the isolation of this fungus from soil in potato cultivation areas during cropping year 2008 in Karaj and Varamain and their biologic control by *T. flavus* under greenhouse conditions (Naraghi *et al*., 2012a), this study was conducted to evaluate them in the field conditions.

MATERIALS AND METHODS

Seed Treatment by *T. flavus* Isolates

- **Selection of the Most Effective Isolates**

Three isolates of *T. flavus*, were selected based on their performances in our previous research studies in which they had shown their effectiveness on the reduction of potato Verticillium wilt disease in laboratory and greenhouse conditions (Naraghi, 2010; Naraghi *et al*., 2012b).

- **Preparation of Inoculum for *T. flavus* Isolates**

According to the results of previous studies, rice bran was the most effective carrier for preparation of the inoculum of *T. flavus* isolates which was used in this study. Each antagonistic isolate was cultured on TF Medium and were preserved for production of ascospore at 30°C for three weeks (Maroise *et al*., 1984). Twenty ml of sterilized distilled water was then added to each Petri plate for washing out the produced ascospores, and preparation of a suspension with 10^6 ascospores ml^{-1} were prepared. Twenty ml of each suspension, was added to each of cellophane bag containing 250 gram of rice bran and mixed thoroughly. The inoculated bags were incubated at 30°C for three weeks for production of enough ascospores. After three weeks, contents of cellophane bags were evacuated and air dried under laboratory conditions

(Naraghi *et al*., 2006).

Preparation of Pathogenic Fugal Inoculum for the Field Experiment

Pathogenic inoculum was prepared for both pathogenic fungi (*V. albo-atrum* and *V. dahliae*) by reproducing them on wheat seeds (Sahayaraj and Raja Namasivayam, 2008). After preparation of the inoculums, the required amount of inoculum was added to the field soil up to 20-cm depth based on twenty kilograms per hectare and concentration (cfu) of less than the threshold level needed for disease occurance (Poudel *et al*., 2001).

The Review on Impact of Seed Treatments Affected by *T. flavus* on Verticillium wilt and Potato Yield in Field

The field experiments were conducted with four treatments and four replications within the statistical design of Randomized Complete Blocks (RCB) during two years (2011- 2012). Treatments included: 1) Seeds affected by the inoculum of isolate TF-Po-V-49; 2) Seed affected by the inoculum of isolate TF-Po-V-50; 3) Seeds affected by the inoculum isolate TF-Po-V-52; 4) The seed affected by Carbendazim fungicide; and 5) Seeds with no inoculum (control).

Each replicate included a plot with four rows each with six meter width and an untreated row between a two plots. To prevent from interference in irrigation, another water ditch was prepared separately rather than wastewater ditch. In this investigation, Draga cultivar (sensitive variety) was used. Treatments were evaluated based on the incidence and severity of disease and growth. Data analysis for each year was conducted separately according to Duncan's multiple- range test using MS TATC statistical software.

Isolation of Verticillium Wilt Pathogenic Agents from the Infected Crops

For isolation of *V. dahliae* and *V. albo-atrum* fungi, the samples of infected root and stem were washed under running tap water. After removing exterior crust, small pieces of trachea tissues of samples were surface disinfested by sodium hypochlorite (5%) for 30-60s and placed in Petri plates containing culture medium of Acidified Potato Dextrose Agar (APDA) amended with 2ml of lactic acid (25%). The plates were stored under temperature 22°C for five to seven days for appearance of Verticillium fungal colonies (Kim *et al*., 2001).

After purification of each fungal colony derived from the surface of culture medium, colonies were subjected to the identified procedure described in the literature (Hawksworth and Tallboys, 1970; Kim *et al*., 2001) based on microscopic and macroscopic characteristics including size of conidium, length of conidiophore, shape of recumbent structures (microsclerotia and dark mycelium) and staining test.

Evaluation of Verticillium Wilt Disease in the Field

To evaluate disease in different treatments, 72 plants were randomly sampled from each plot one month before harvest and based on the symptoms of Verticillium wilt disease, the severity of disease was determined based on the scale of 0-4 according to Susnoschi *et al*. (Susnoschi *et al*., 1976) procedure as follows:

0: without symptom **Scale 1:** Symptoms as green and yellow colors with wilt limited to lower leaves

Scale 2: Symptoms as slightly yellow color and wilt in some leaves **Scale 3:** Symptoms as extremely yellowish color and wilt in some leaves and stem

Scale 4: Completely wilted plant

Thus, the severity of disease was determined in different treatments after calculation of average disease severity in 72 plants. Similarly, percentage of disease incidence in each plot was determined based on the number of plants with wilt

symptoms among 72 sampled plants and consequently percentage of disease incidence was calculated for all treatments. To evaluate symptoms of Verticillium wilt, method described by Stevenson *et al*. (Stevenson *et al*., 2001) was used as follows:

Verticillium wilt initially started from lower organs of the plant and gradually advanced to upper parts so that occasionally only leave at the top of plant remained green. Wilted leaves first became pale yellow and later turned brown. The primary symptoms of disease appeared as yellow color on one side of the leaves and or stem. The infected plants then wilted, destroyed rapidly and or slowly depending on disease severity where tuft and burning at the end of bracts were visible in the infected plants.

RESULTS

Preparation of Treatments Affected by Isolates of *T. flavus*

- **Selection of Isolates of *T. flavus***
  TF-Po-V-49, TF-Po-V-50, and TF-Po-V-52 were selected as the most effective isolates of *T. flavus*.

- **Preparation of Inoculum of *T. flavus* Isolates**

In order to treat 10kg of potato seeds with the inoculums of selected *T. flavus* isolates, for each treatment, *T. flavus* inoculum was prepared with the weight of twice as potato seeds weights.

Determination of Colony Formating Units (CFU) for Verticillium Wilt Agents in the Field Soil

Average number of CFU per one gram field soil was calculated as 16.33. Thus, due to lower quantity of the given amount compared to the needed threshold level for occurrence of Verticillium wilt disease, pathogenic inoculum was prepared for using in the field.

Preparation of Pathogenic Inoculum for Application in the Field

One kilogram of pathogenic inoculum was used in the field experiment with the area of 500 square meter on the base of recommended dose of 20kg per hectare (Poudel *et al*., 2001).

Isolation of Pathogenic Agents of Verticillium Wilt from Infected Plants in the Field

The results obtained from macroscopic and microscopic examinations on *V. dahliae* and *V. albo-atrum* isolated from the infected plants indicated that these isolates had conidium with the size of 2.5-10.2×2.3-3.5 and 2.5-8.6×2.0-3.0 µm respectively; while length of conidiophore in *V. albo-atrum* was greater than in *V. dahliae*. Likewise, in *V. dahliae*, microsclerotia were seen but in *V. albo-atrum* dark mycelium was observed. The color of rear and front parts of *V. dahliae* and *V. albo-atrum* colonies appeared transparent (hyaline) to black and transparent through grayish white respectively when cultured on PDA culture medium.

Evaluation of Verticillium Wilt Disease and Yield in the Field

- **Year 2011**

In farming year 2011, evaluation of the impact of seed treatments affected by *T. flavus* isolates and Carbendazim fungicide on Verticillium wilt disease was significant at 1% probability level. According to the statistical analysis of the results, treatments were divided into three statistical groups (Table 1). The minimum disease incidence (6.94%) was observed in the treatment affected by Carbendazim fungicide. Similarly, the maximum disease incidence (13.19%) belonged to control treatment (Table 1). On the other hand, reduction of disease was observed in all treatments affected by

antagonistic fungal isolates compared to the control and among them treatments TF-Po-V-50 and TF-Po-V-52 with 7.98 and 7.28% of disease showed the more efficiency in reduction of disease compared to variety TF-Po-V-49 (10.41% of disease) (Table 1).

According to the the results, the impact of seed treatments affected by isolates *T. flavus* and Carbendazim fungicide on the severity of Verticillium wilt disease in potato was significant at 5% probability level. In terms of infection index, there was no significant difference among treatments containing TF-Po-V-49, TF-Po-V-50, and TF-Po-V-52 with Carbendazim, but disease severity average was significantly reduced in all the above-mentioned treatments as 1.17, 0.75, 0.85, and 1 respectively, compared to control (2.20) (Table 1).

Results also indicated that the effect of seed treatments affected by *T. flavus* isolates and Carbendazim on yield of potato was significant at 5% probability. Statistical analysis of the yield aveage values, divided them into four statistical groups. Among all treatments, the maximum average value of yield (34.86 tons per hectare) belonged to treatment affected by Carbendazim fungicide while TF-Po-V-52 treatment with yield value of 32.33 ton/hectare was ranked at the second position. Likewise, the minimum yield value (25.26 ton/hectare) was observed in control (Table 1). There was no significant difference among the treatments affected by antagonistic isolates of TF-Po-V-49 and TF-Po-V-50 in terms of average yield (Table 1).

- **Year 2012**

In farming year 2012, there was no significant difference among treatments in terms of all measured factors including disease incidence, disease severity and yield. The results of second year showed that the significant difference among disease and yield average in two treatments of TF-Po-V-49 and control one with mean of the given attributes in all factors played major role in the presence of significant difference among treatments in this test (Table 2). **Combined analysis of the Results of Two Years**

The results of combined data analysis obtained from two farming years showed that difference among treatments was significant in terms of disease incidence and severity at 5% probability level and in terms of yield at 1%. Similarly, there was a significant reduction in disease severity in all treatments compared to control while no significant difference was observed between treatments affected by antagonistic isolates and treatment contained Carbendazim in terms of disease severity (Table 3).

According to these results, disease incidence in all treatments has was reduced in comparison with the control with the exception of treatment TF-Po-V-49, there was no significant difference among other treatments (TF-Po-V-50, TF-Po-V-52, and Carbendazim) (Table 3). Statistical analysis of the results in terms of average yield also indicated that in all treatments yield was increased 3.69-26.27% and among them the treatments affected by Carbendazim and TF-Po-V-52 had the highest increase as 28.74 and 26.74 ton/hectare respectively in comparison with the control (22.76 Ton/ha) (Table 3).

DISCUSSIONS

The overall results of this study showed that it was possible to control Verticillium wilt disease caused by *V. dahliae* and *V. albo-atrum* using antagonist fungus (*T. flavus*) in potato fields. In this study and based on the results of our previous study (Naraghi, 2010), there effective isolates of *T. flavus* were used which were initially isolated from potato fields in Varamin area.

In the present research, fungal antagonists which were obtained from Varamin and Hamedan regions showed different efficacy which could probably be related to their growth temperature requirement and their responses to the potato root (rhizome) exudates. Concerning the above-mentioned, subjects, there have been several reports about optimal growth of *T. flavus* isolates within range of temperature 30-40°C and occupation of rhizosphere of some crops including cotton, eggplant, potato, and tomato by fungus and the role of root exudations of these plants (Marois *et al*., 1984; Duo- Chuan *et al*., 2005).

Inceoglu *et al*. (2012) during their study about the impact of soil and plant cultivars on bacterial microorganisms on potato rhizosphere, reported that presence of glucose compounds in root exudations played important role in these interactions. Alternatively, Antagonistic effects of *T. flavus* depend on glucose oxidase that its activity has been proved with the presence of glucose (Kim *et al*. 1990; Fravel & Robert, 1991). In the current study, in addition to isolation of potato verticillium wilt pathogenic agents (*V. dahliae* and *V. albo-atrum*), the population of their propagules number in the field soil as about 20 CFU/ gr was also calculated. In a previous study, the populations of *V. dahliae* and *V. albo-atrum* in potato fields in Hamedan, Tehran, Khorasan, Kerman, Kurdistan, Ardebil, and Isfahan provinces have been reported as about 33 CFU per gram of soil (Mansoori *et al*., 2006).

Likewise, the field results in the present research in the farming year of 2011 showed that all treatments affected by antagonistic isolates of *T. flavus* (TF-Po-V-49, TF-Po-V-50, and TF-Po-V-52) and Carbendazim fungicide reduced significantly in terms of the disease incidence and severity significantly and also increased the yield in comparison with the untreated control. This result was complied with the findings of various studies regarding biological or chemical control of potato Verticillium wilt disease by means of antagonist fungi like *Thrichoderma atroviride* and Carbendazim fungicides, or Captan and Benomyl under field conditions (Fravel, 1989; Bruner *et al*., 2005; Goicoechea, 2009).

The results of the year 2011 indicated that there was no significant difference among the affected treatments by antagonists with Carbendazim treatment in terms of incidence and severity of disease while a significant difference was observed between treatments in terms of yield and the maximum yield belonged to Carbendazim and TF-Po-V-52 treatments. With respect to lack of significant difference among the affected treatment by Carbendazim and TF-Po-V-52 in terms of disease incidence and severity and the promoted germination ability in several treatments containing *T. flavus* isolates, it can be concluded that inadequate quantity of CFUs in the test antagoniats can affect and result in their inadequate antagonistic performances. The findings of the conducted studies on the manipulation of soil inhibitor and improvement of the population of antagonists has previously been shown indicating that this class of soils has been extremely effective in reduction of soil-borne pathogenic agents and increasing the yield (Alabouvette, 1989; Fernando *et al*., 2004).

Alternately, observation of the existing difference among TF-Po-V-52 isolate from TF-Po-V-49 and TF-Po-V-50 isolates in terms of efficacy to increase yield may be related to several factors including the quantity and quality of produced metabolites due to genetic variation (Madi *et al*., 1992). According to the results of second field study in 2012, there was significant difference among different treatments at probability level of 5% in terms of evaluated criteria. With respect to implementation of project in the framework of randomized complete blocks, it was possible in some blocks that due to land lope, the accumulation and density of plants humus, result in inoculum of *T. flavus* became further by irrigation and this might cause inequality of the efficacy of *T. flavus* inoculum remained from previous year. In a similar previous study on the biological control of fusarium wilt on bean root using Thrichoderma isolates, the above-mentioned phenomenon took place (Muriungi *et al*., 2013).

Similarly, during year 2012, there was no significant difference among the experimental treatments in terms of disease incidence and severity and yield. Comparison of the results in two farming years showed that in the first year, the significant difference in terms of disease and yield in two treatments of TF-Po-V- 49 and control played a remarkable role in the presence of significant relationship between the tested treatments. In the second year, however, the disease and yield in both above-mentioned treatments with reduction in disease (26.51 and 30.98%) and increase in yield (66.35 and 78.14%) approached to average of these attributes in all treatments while they showed no significant difference from other treatments.

Therefore, In the conducted experiment in year 2012, it is concluded that due to the presence of the remaining potato rhizomes affected by *T. flavus* inoculum within the soil , employing of seed treatments by *T. flavus* led to an improvment in the population of these isolates in all treatments and lack of significant difference among them was measured in terms of all criteria . In similar studies, promotion of population of microorganisms in the soil of potato fields by application of affected treatments from antagonistic fungi has been reported (Lodhi, 2004; Berg *et al*., 2005).

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APPENDICES

Table 1: Comparison of Verticillium Wilt Disease Incidence, Disease Severity and Yield of Potato in Different Treatments Affected by Isolates of *Talaromyces flavus* and Carbendazim Fungicide during Year 2011

Treatment	Disease Incidence (%)	Disease Severity Index	Yield (Ton/Ha)
TF-Po-V-49	10.41ab **	1.17b *	26.99 bc *
TF-Po-V-50	7.98b	0.75b	27.78 bc
TF-Po-V-52	7.28b	0.87b	32.33ab
Carbendazim	6.94b	1.00b	34.86a
Control	13.19a	2.20a	25.26c

Values marked with the same letter (s) in the columns are not statistically different according to Duncan's Multiple Range Test ($p > 0.01$)

Values marked with the same letter (s) in the columns are not statistically different according to Duncan's Multiple Range Test ($p > 0.05$).

Table 2: Comparison of Verticillium Wilt Disease Incidence Disease Severity and Yield of Potato in Different Treatments Affected by Isolates of *Talaromyces flavus* and Carbendazim Fungicide during Year 2012

Treatment	Percent Disease Incidence	Disease Severity Index	Yield (Ton/Ha)
TF-Po-V-49	7.65a*	1.03a*	44.90a*
TF-Po-V-50	5.97a	1.02a	44.55a
TF-Po-V-52	6.60a	0.98a	47.00a
Carbendazim	5.55a	0.99a	50.30a
Control	10.07a	1.08a	45.00a

Values marked with the same letter (s) in the columns are not statistically different according to Duncan’s Multiple Range Test (p > 0.05).

Table 3: Comparison of Different Treatments during Years 2011 and 2012 in Terms of Disease Incidence, Disease Severity and Total Yield by Application of Combined Data Analysis

Treatment	Disease Percentage	Disease Intensity	Yield (Ton/Ha)
TF-Po-V-49	9.03ab**	1.28b*	23.60bc**
TF-Po-V-50	6.97b	1.02b	23.92bc
TF-Po-V-52	6.94b	1.08b	26.74ab
Carbendazim	6.24b	1.02b	28.74a
Control	11.63a	2.12a	22.76c

Values marked with the same letter (s) in the columns are not statistically different according to Duncan’s Multiple Range Test (p > 0.05).